AVIAN DISEASES 50:572–578, 2006

Molecular and Biological Characterization of a Naturally Occurring Recombinant Subgroup B Avian Leukosis Virus with a Subgroup J–Like Long Terminal Repeat

Blanca Lupiani,[AB] Arun R. Pandiri,[B] Jody Mays,[C] Henry D. Hunt,[C] and Aly M. Fadly[C]

[A]Department of Veterinary Pathobiology, College of Veterinary Medicine,
Texas A&M University, College Station, TX 77843
[B]Department of Pathobiology and Diagnostic Investigation, College of Veterinary Medicine,
Michigan State University, East Lansing, MI 48824
[C]U.S. Department of Agriculture, Agricultural Research Service, Avian Disease and Oncology Laboratory,
3606 East Mount Hope Road, East Lansing, MI 48823

Received 25 May 2006; Accepted 25 July 2006

SUMMARY. Infection of broiler chickens with subgroup J avian leukosis virus (ALV) results in the induction of myeloid tumors. However, although egg-type chickens are susceptible to infection with ALV-J, the tumor incidence is very low, and on rare occasions the tumors observed are of the myeloid lineage. We recently described the isolation of an ALV (AF115-4) from commercial egg-type chickens suffering from myeloid leukosis. AF115-4 was initially identified as an ALV-J isolate based on PCR analysis of the long terminal repeat (LTR). However, further characterization of the viral envelope indicated that the virus is recombinant with subgroups B envelope and J LTR. Here we further characterize this recombinant virus at both the molecular and biological levels. We show that the AF115-4 isolate expresses a recombinant envelope glycoprotein encoded by a subgroup B gp85 region and a subgroup E gp37 region. The host range of AF115-4 was analyzed using cells resistant to infection by subgroups A/B, J, or E; this shows that no ALV-J was present in the isolates obtained from the affected chickens. Additional antigenic characterization of AF115-4 using chicken sera specific for subgroups B or J indicated that no ALV-J was present in the samples examined. Inoculation of AF115-4 into ALV-susceptible $15I_5 \times 7_1$ chickens resulted in the induction of lymphoid leukosis but not the expected myeloid leukosis affecting the commercial chickens. These results suggest that differences in the genetic makeup of the chickens from which AF115-4 was isolated and the line $15I_5 \times 7_1$ used in the present experiments may be responsible for the observed differences in pathogenicity. In addition, the results suggest that ALV-J continues to evolve by recombination, generating new viruses with different pathological properties.

RESUMEN. Caracterización biológica y molecular de un virus recombinante de leucosis aviar subgrupo B de ocurrencia natural, con terminaciones repetidas largas del subgrupo J.

La infección de pollos de engorde con el virus de leucosis aviar subgrupo J resulta en la inducción de tumores mieloides. Sin embargo, aunque las aves de las líneas ponedoras son susceptibles a la infección con el virus de la leucosis aviar subgrupo J, la incidencia de tumores es muy baja y en muy escasas ocasiones los tumores observados son de la línea mieloide. Recientemente describimos el aislamiento de un virus de leucosis aviar (AF115-4) proveniente de líneas de ponedoras que tenían leucosis mieloide. Basado en el análisis de sus terminaciones repetidas largas mediante la prueba de reacción en cadena por la polimerasa, el aislamiento AF115-4 fue identificado inicialmente como un aislamiento de leucosis aviar subgrupo J. Sin embargo, caracterizaciones adicionales de la envoltura viral indicaron que la cepa viral es una recombinante que presenta la envoltura de los subgrupos B y las terminaciones repetidas largas del subgrupo J. En el presente trabajo se caracteriza más a fondo este virus recombinante tanto a nivel molecular como a nivel biológico. Se demostró que los aislamientos AF115-4 expresan una glicoproteína de envoltura recombinante codificada por una región gp85 perteneciente al subgrupo B y una región gp37 perteneciente al subgrupo E. Se analizó el rango de huéspedes del aislamiento AF115-4 utilizando células resistentes a la infección con los subgrupos A/B, J, o E; mostrando que el virus de leucosis aviar subgrupo J no estaba presente en ninguno de los aislamientos obtenidos de las aves afectadas. Caracterizaciones antigénicas adicionales del aislamiento AF115-4 utilizando antisuero específico para los subgrupos B o J, demostraron que el virus de leucosis aviar subgrupo J no estaba presente en las muestras examinadas. La inoculación de aves susceptibles al virus de leucosis aviar ($15I_5 \times 7_1$) con el aislamiento AF115-4 resultó en la inducción de leucosis linfoide, pero no en la esperada leucosis mieloide que afecta las aves comerciales. Estos resultados sugieren que diferencias en la constitución genética de las aves de donde se aisló el virus AF115-4 y la línea genética utilizada en estos experimentos ($15I_5 \times 7_1$), deben ser las responsables de las diferencias observadas en la patogenicidad. Adicionalmente, los resultados sugieren que el virus de leucosis aviar subgrupo J continúa evolucionando mediante recombinación, generando nuevos virus con diferentes propiedades patológicas.

Key words: avian leukosis virus (ALV), lymphoid leukosis, myeloid leukosis, pathogenesis, recombinant ALV

Abbreviations: ALV = avian leukosis virus; CEF = chicken embryo fibroblast; EAV = endogenous avian retrovirus; ELISA = enzyme-linked immunosorbent assay; LL = lymphoid leukosis; LTR = long terminal repeat; ML = myeloid leukosis; PCR = polymerase chain reaction; PI = postinoculation; SU = surface domain; TM = transmembrane domain; UTR = untranslated region

Avian leukosis virus (ALV) infection of commercial poultry has a global distribution. Losses associated with ALV infection, estimated in millions of dollars each year, are due to neoplastic-associated mortality and poor performance in subclinically infected chickens. ALVs isolated from chickens have been classified into six subgroups (A, B, C, D, E, and J) based on their viral envelope, which in turn determines host range, cross-neutralization, and viral interference (5). ALVs of chickens are further classified as exogenous (A, B, C, D, J) or endogenous (E) based on their mode of transmission. Exogenous viruses are transmitted horizontally or vertically, while

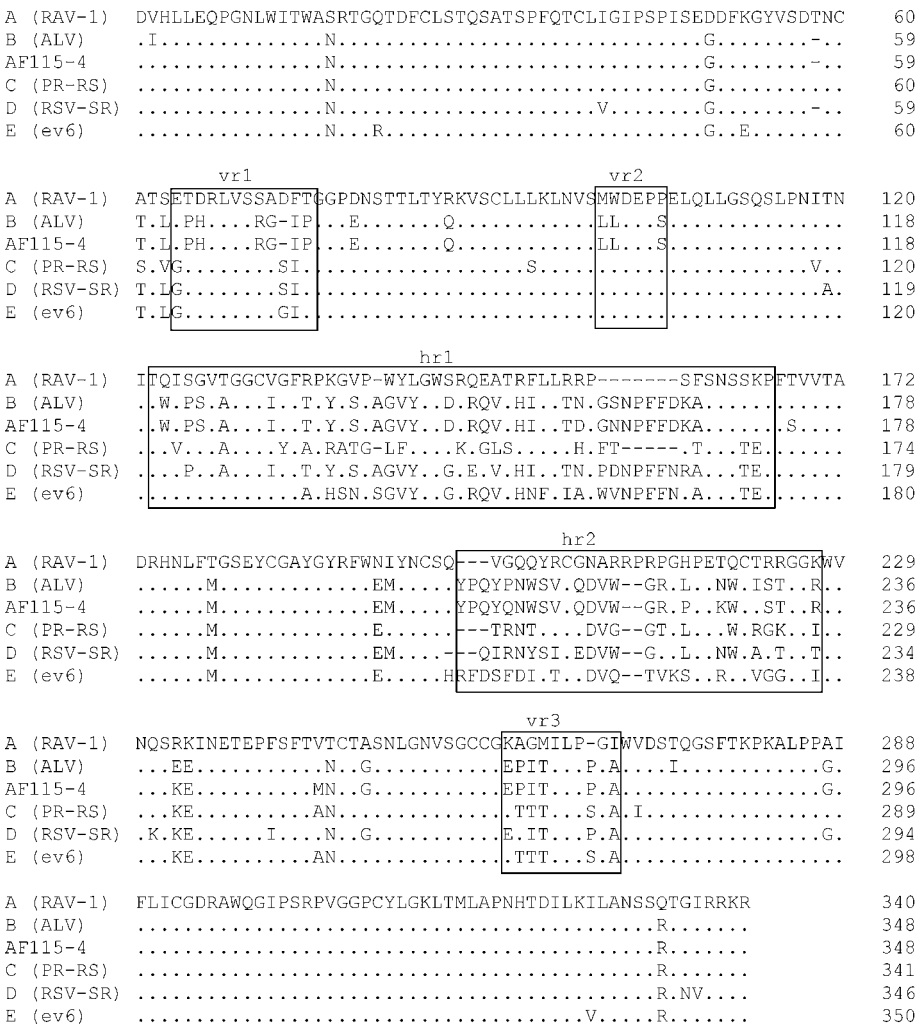

Fig. 1. Sequence alignment of the deduced sequence of gp85 protein of AF115-4 and the representative isolates of ALV A-E: A (RAV-1) (Lupiani, unpubl. data); B (ALV) (AF507033); C (Pr-RSV) (X51862); D (RSV-SR) (BAD98245); E (*ev6*) (AY013305). Variable (vr) and hypervariable (hr) regions are boxed.

endogenous viruses are transmitted primarily through the germline but can occasionally transmit horizontally and vertically (20). Exogenous viruses are considered highly oncogenic, while endogenous viruses induce little or no pathogenicity. Subgroups A and B are the most common ALVs found in egg-type chickens and are associated with lymphoid leukosis (LL), while subgroup J, which appeared in 1988 in the United Kingdom, is mainly associated with myeloid leukosis (ML) in meat-type chickens. During the 1990s, the disease associated with ALV-J became one of the most important disease problems facing the broiler industry, causing devastating losses worldwide (29). Although control of ALV has been achieved mainly by elimination of infected dams from breeding stocks (26), eradication of ALV-J has been difficult because of significant genetic and antigenic variation observed among ALV-J isolates (10,14,24,30) and high levels of vertical and horizontal transmission (29).

Retroviruses have been shown to have a high mutation rate due to the activity of the reverse transcriptase enzyme (8). In addition, the diploid nature of the retrovirus genome results in a high level of recombination (12,13,19). Recombination has been observed between exogenous viruses, exogenous and endogenous viruses (15), and exogenous viruses and nonhomologous cellular genes (27). Initial sequence analysis of the ALV-J genome indicated low similarity of the *env* gene to that of previously described ALV subgroups (40%), but surprisingly, a high level of similarity (95%–97%) to members of the ancient endogenous virus family (ev/J, endogenous avian retrovirus (EAV)-HP, and Line 0 *env*-like) (2,3,24,25). These data led to the hypothesis that ALV-J arose by recombination between an exogenous ALV and an EAV. Recently we described the isolation and characterization of recombinant viruses between two ALV-J isolates and a defective recombinant endogenous virus present in the transgenic chicken line *alv6* (16). These recombinant viruses had acquired the *env* gene of subgroup A and maintained the long terminal repeat (LTR) of ALV-J. Biologically, these viruses induced primarily LL in layers (17); however, we were unable to determine their pathogenicity in commercial broilers because they lack the subgroup A receptor necessary for infection (Lupiani, unpubl. data). On the other hand, we recently described the isolation of an ALV associated with ML in a layer flock. Although initial characterization suggested this virus isolate was similar to subgroup J, further sequence analysis of the viral envelope revealed that it was similar to subgroup B (11). In the present report we present data on sequence analysis of the viral *env* gene, 3′ untranslated region (3′UTR) and LTR as well as the host range, antigenicity, and *in vivo* characterization of this virus.

A	B	C	D	E	AF115-4	Virus
	77.9	82.9	80.6	81.8	78.2	A
		78.6	87.9	80.5	96.8	B
			83.0	85.9	78.9	C
				84.1	87.3	D
					81.0	E
						AF115-4

Fig. 2. Amino acid percentage identity among gp85 proteins of AF115-4 and representative strains of different ALV subgroups.

MATERIALS AND METHODS

Viruses. Plasma samples from commercial white egg-type chickens suffering from myeloid leukosis (AF115-4) (11) were inoculated onto the line 0 (C/E) chicken embryo fibroblasts (CEFs) and incubated at 37 C. Seven days postinoculation (PI), Tween 80 (0.2% final concentration) was added to the cells, the plates were freeze-thawed twice, and 100 µl of the resulting lysates was tested for the presence of ALV group-specific antigen (p27) protein by an enzyme-linked immunosorbent assay (ELISA) (16). RAV-1 (subgroup A), RAV-2 (subgroup B), and HC-1 (subgroup J) viruses were used as controls.

PCR cloning and sequencing. To characterize the ALV isolated (AF115-4), the viral envelope gene, 3′ untranslated region, and most of the 3′ LTR were PCR amplified and sequenced. Briefly, proviral DNA was isolated from line 0 CEF infected with plasma samples of affected chickens using the Puregene DNA isolation kit (GENTRA Systems Inc., Minneapolis, MN) according to the manufacturer's instructions. PCR was carried out in 25 µl reactions with Cloned PFU polymerase (Stratagene, La Jolla, CA) and 100 ng of DNA. Reactions without DNA and with DNA from uninfected line 0 CEF were used as controls. The primers used were as follows: "7J" (forward) 5′ CCC AAA AGG ATG AGG TGA CTA AGA 3′ and "S2" (reverse) 5′ AGT TGT CAG GGA ATC GAC 3′. The PCR conditions were as follows: denaturation at 94 C for 4 min; 25 cycles consisting of denaturation at 94 C for 1 min, annealing for 1 min at 56 C and extension for 2 min at 72 C; and a final cycle of 72 C for 7 min. PCR products were gel purified using Qiaex II (Qiagen, Santa Clarita, CA) and cloned into pCR-Blunt plasmid (Invitrogen, Carlsbad, CA) following the manufacturer's instructions. Positive plasmid clones were sequenced using an ABI Model 373A automatic DNA sequencer (Applied Biosystems, Foster City, CA). The sequences obtained were analyzed using Sequencher Version 3.1 (Gene Codes Corporation, Ann Arbor, MI), DNAstar (DNAStar Inc., Madison, WI), and the BLAST program from NCBI (www.ncbi.nlm.nih.gov). Because of the possibility of genetic variation, three independent clones were sequenced.

Cells. The host range of the AF115-4 was determined using CEF from chicken lines with different subtype susceptibility. Viral stocks obtained from line 0 CEFs were inoculated onto line 0 (resistant to subgroup E infection) and 7_2 (resistant to subgroups A and B infection) CEFs and DF-1 (resistant to subgroup E infection) and and DF-1/J (resistant to subgroups E and J infection) immortalized embryo fibroblast cell lines (14). Because of the presence of endogenous viruses in line 7_2 CEFs, supernatants from all infected cells were passed one more time on line 0 CEFs prior to detection of viral group–specific antigen by ELISA (16).

Flow cytometry. To confirm the results obtained by cell culture analysis and sequencing, reactivity of AF115-4 virus with antibodies specific for RAV-2 (subgroup B) and HC-1 (subgroup J) was examined

```
A (RAV1)     SHLDDTCSDEVQLWGPTARIFASILAPGVAAAQALREIERLACWSVKQANLTTSLLGDLL    60
B (RSV-SR)   ..................................K.......................    60
C (Pr-RSV)   .....................................P.....................    60
D (RSV-PR)   ............................................................    60
E (ev6)      ............................................................    60
AF115-4      ............................................................    60
J (4817)     .R.SPD....I...SA.......FF..........K................SLI.NAM.    60

A (RAV1)     DDVTSIRHAVLQNRAAIDFLLLAHGHGCEDIAGMCCFNLSDHSESIQKKFQLMKEHVNKI   120
B (RSV-SR)   ............................V...........................    120
C (Pr-RSV)   ............................V.......................K.....    120
D (RSV-PR)   N...........................V.........N...................    120
E (ev6)      ............................V...........................    120
AF115-4      ............................V...........................    120
J (4817)     E.MN...................Q....Q.VE..............H.AL.A....TE..    120

A (RAV1)     GVDNDPIGSWLRGLFGGIGEWAVHLLKGLLLGLVVILLLVVCLPCLLQ----CVSSSIRK   176
B (RSV-SR)   ...S.....................................................I.CGN...    176
C (Pr-RSV)   ...S.........I.....................................----F.......    176
D (RSV-PR)   ...S.....................................................I.CG....    176
E (ev6)      ...S.....................................................I.......    176
AF115-4      ...S.........I.....................................----........    176
J (4817)     R.ED....D.FTRT..SF.G.LAKGV.T.MFA.F..VC.LAII..IIKCFQD.L.RTMYQ   180

A (RAV1)     MIDNSLGYREEYKKLQEAYKQPERRA                                     202
B (RSV-SR)   ..N..IS.HT......K..G...S.IV                                    203
C (Pr-RSV)   ..NS.IN.HT..R.M.GGAV                                           196
D (RSV-PR)   ..N..IS.HT......K.CG...S.IV                                    203
E (ev6)      ..N..IS.HT..Q...K.CR...NG.V                                    203
AF115-4      ..N..IS.HT......K.CR...NG.V                                    203
J (4817)     FM.ERIS.HA......K.CR...NG.V                                    207
```

Fig. 3. Sequence alignment of the gp37 envelope protein of the different AF115 isolates and representatives of ALV A-J: A (RAV-1) (Lupiani, unpubl. data); B (ALV) (AF507033); C (Pr-RSV) (X51862); D (RSV-SR) (BAD98245); E (*ev6*) (AY013305); J (4817) (AF247385).

Table 1. Infectivity of recombinant AF115-4 virus in cells of different phenotypes.

Cells	Virus			
	AF115-4	ALV-A	ALV-B	ALV-J
Line 0 (C/E)	+	+	+	+
7_2 (C/AB)	−	−	−	+
DF-1 (C/E)	+	+	+	+
DF-1/J (C/EJ)	+	+	+	−

by flow cytometry. Briefly, uninfected and AF115-4, RAV-2, and HC-1 infected and DF-1 cells were trypsinized and resuspended in phosphate-buffered saline supplemented with 1% bovine serum albumin and 0.1% sodium azide (staining medium) at a final concentration of 10^7 cells/ml. Chicken convalescent sera to RAV-2 and HC-1 were diluted to 1:50 in staining medium, added to 100 μl of cells, and incubated for 20 min at 4 C. Cells were washed three times with staining medium and incubated with goat anti-chicken (1:500) labeled with fluorescein isothiocyanate (Bethyl Laboratories, Montgomery, TX) for 15 min at 4 C. Cells were washed three times as described above and resuspended in staining medium containing 1 mg/ml propidium iodide, and 10,000 viable cells were analyzed using a FACSort instrument and Cellquest software (Becton Dickinson, Mountain View, CA).

***In vivo* characterization of the ALV isolate.** Chickens used in the present studies were ALV-susceptible F1 progeny ($15I_5 \times 7_1$) of the Avian Disease and Oncology Laboratory (U.S. Department of Agriculture, East Lansing, MI) line $15I_5$ males and line 7_1 females (1). One-day-old $15I_5 \times 7_1$ chickens were inoculated intra-abdominally with 10^3 tissue culture infectious dose ($TCDI_{50}$/ml) units of AF115-4, RAV-2, and HC-1. Each group consisted of 25 chickens that were kept in isolation until termination at 30 wk of age. At 2, 4, 10, 19, and 30 wk PI, chickens were tested for viremia and shedding as previously described (17). Chickens were also observed for tumors until 30 weeks of age.

Pathology. Chickens that died and those that survived the experimental period were necropsied. Samples of affected tissues were fixed in 10% buffered formalin, stained with hematoxylin and eosin, and examined for microscopic lesions. The type of tumors induced by the viruses was determined based on characteristic gross and microscopic lesions.

Statistical analysis. Data were analyzed using the chi-square statistic test of the SAS statistical software for Windows, version 8.2 (SAS Institute Inc., Cary, NC).

RESULTS

We have recently described the isolation of an ALV (AF115-4) from commercial white egg-type chickens suffering from myeloid leukosis (11). Detailed molecular characterization of the viral envelope gene, 3′ UTR and LTR of this virus was carried out by sequence analysis. PCR of line 0 CEF infected with the ALV AF115-4 isolate using primers upstream of the *env* gene and in the U5 region of the LTR resulted in an amplicon of 2,455 bp.

A	B	C	D	E	J	AF115-4	Virus
	92.1	90.8	91.6	93.1	61.4	93.6	A
		91.8	97.5	95.1	62.6	94.6	B
			91.8	93.9	58.7	94.4	C
				95.6	62.1	95.1	D
					64.5	98.5	E
						65.5	J
							AF115-4

Fig. 4. Amino acid percentage identity among gp37 proteins of AF115-4 and representative strains of different ALV subgroups.

Sequence analysis confirmed that this PCR product encodes the *env* gene of the ALV isolate. Detailed analysis of the deduced amino acid sequence of the surface (SU) glycoprotein (gp85) indicates that it belongs to subgroup B (96.8% identity and 98% similarity) family of ALV (Figs. 1 and 2). Further analysis of the deduced amino acid sequence of the transmembrane (TM) glycoprotein (gp37) indicates that it has 98.5% identity with subgroup E (ev6) but only 94.6% identity with the gp37 protein of subgroup B (Figs. 3 and 4). Interestingly, sequence analysis of the 3′ UTR (from the stop codon of the *env* gene) and LTR indicated the highest identity (94%) with the same region of the ALV-J isolate HPRS-103 including a complete copy of the E element (Fig. 5). The only significant difference observed between these two sequences was an 11 nucleotide deletion in the U3 region of AF115-4.

To rule out the possibility of the presence of an ALV-J that was not PCR amplified with the primer set used, the host range of the virus present in the original AF115-4 sample was determined. Primary CEFs from chicken lines known to be resistant to subgroups A and B (7_2) as well as the continuous cell line DF-1/J cell, which is resistant to ALV-J infection, were inoculated with virus stocks obtained from line 0 CEFs (sensitive to all ALV subgroups except E) inoculated with the original AF115-4 sample. As seen in Table 1, no virus was detected in line 7_2 CEFs infected with AF115-4, while virus was detected in DF-1/J cells, indicating that no ALV-J was present in the samples obtained from the affected chickens.

To confirm the results obtained by sequence analysis and host range, DF-1 cells infected with AF115-4 were examined by flow cytometry using chicken polyclonal serum specific for subgroups B and J. Results show that the AF115-4 isolate reacted with the subgroup B but not with the subgroup J specific antiserum, confirming that no ALV-J was present in the samples examined (data not shown).

Table 2. Virus isolation from plasma (V) and cloacal washes (CS) of $15I_5 \times 7_1$ chickens inoculated with AF115-4, ALV-J (HC-1), and ALV-B (RAV-2).[A]

Group	Week 2		Week 4		Week 10		Week 19		Week 30	
	V	CS	V	CS	V	CS	V	CS	V	CS
Control	0/25[Ba]	0/25[a]	0/25[a]	0/25[a]	0/25[a]	0/25[a]	0/25[a]	0/25[a]	0/25[a]	0/25[a]
AF115-4	18/25[b]	7/25[b]	23/25[b]	20/25[b]	13/22[b]	18/22[b]	16/22[b]	19/22[b]	13/15[b]	11/15[bc]
ALV-J	22/25[b]	3/25[c]	25/25[b]	25/25[b]	2/24[c]	9/22[c]	18/24[b]	7/24[c]	19/23[c]	9/23[c]
ALV-B	22/25[b]	2/25[c]	25/25[b]	24/25[b]	12/25[b]	22/25[b]	13/19[b]	18/19[b]	9/13[b]	12/13[c]

[A]Within each weekly column the percentages among experimental groups with different lowercase letters differ significantly based upon chi-square analysis ($P < 0.05$).

[B]Number of infected chickens/number of chickens in group.

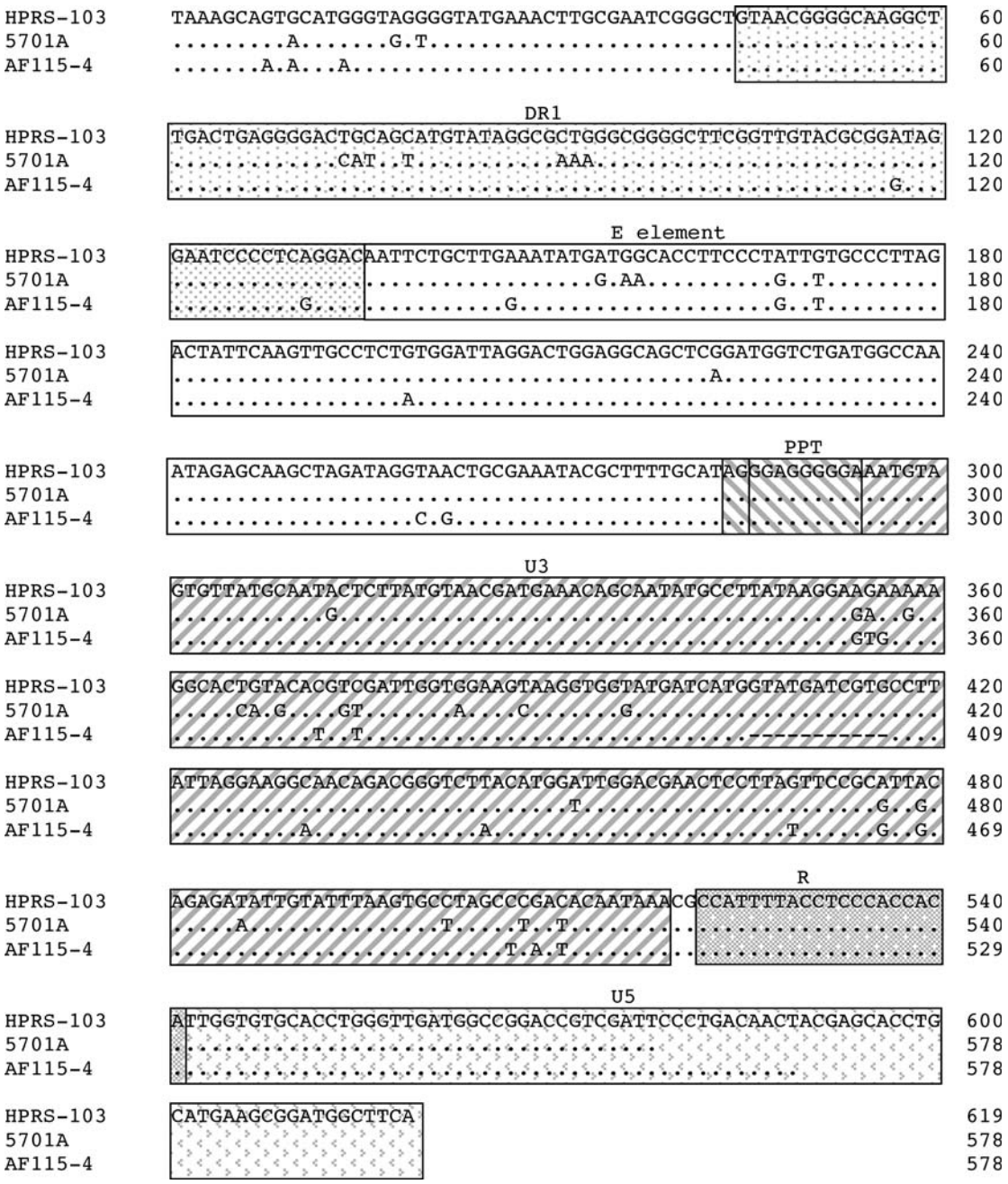

Fig. 5. Sequence alignment of the 3′ untranslated region and LTR of HPRS103 (ALV-J) (Z46390), 5701A (ALV-A/J) (AF257655), and AF115-4. Different regions of 3′ UTR and LTR are indicated: direct repeat (DR1), E element, polypurine track (PPT), unique region 3 (U3), repeat region (R), unique region 5.

In vivo characterization of the AF115-4 virus was carried out in the experimental egg-type chicken line $15I_5 \times 7_1$. RAV-2 and HC-1, subgroups B and J representative strains respectively, were used as controls. Two, 4, 10, 19, and 30 weeks PI plasma and cloacal swab washes were taken and examined for presence of virus by virus isolation and antigen capture ELISA specific for the p27 protein. All inoculated chickens, with the exception of two in the AF115-4 group, were positive for virus in both plasma and cloacal swab washes at least at one of the time points tested. Most of the chickens presented either constant or transient viremia with samples being positive regardless of the virus used (Table 2). Unexpectedly, the tumors induced by AF115-4 virus were mostly LL, similar to those obtained with the subgroup B control (Table 3). No ML tumors were detected in any of the chickens regardless of the virus used. No significant differences were seen in the incidence of LL observed between AF115-4 (8 %) and ALV-B (11%), although the difference was significant between these two groups and ALV-J (0%). On the other hand, the incidence of other tumors (rhabdomyosarcoma, hemangioma, stem cell tumor, erythroblastosis) was not significant between AF115-4 (8%) and ALV-J (12%) but was significant between these two groups and ALV-B (36%). Significant differences were observed in the total incidence of tumors among the three experimental groups examined.

DISCUSSION

Subgroup J ALV arose by recombination between an unknown exogenous ALV and a member of the EAV-J family of ancient

Table 3. Incidence and type of tumors induced by AF115-4 virus in the experimental $15I_5 \times 7_1$ chicken line.

Group	Tumor type and incidence[AB]			
	LL	ML	Other	Total chickens
None	0/25 (0)[a]	0/25 (0)[a]	0/25 (0)[a]	0/25 (0) [a]
AF115-4	8/25 (32)[b]	0/25 (0)[a]	2/25 (8)[b,C]	9/25 (36)[b]
ALV-J	0/25 (0)[a]	0/25 (0)[a]	3/25 (12)[b]	3/25 (12)[c]
ALV-B	11/25 (44)[b]	0/25 (0)[a]	9/25 (36)[c,D]	18/25 (72)[d]

[A]Number of positive chickens/number chickens in each group (percentage).

[B]Within each column, the percentages of tumor incidence among experimental groups with different lowercase letters differ significantly based upon chi-square analysis ($P < 0.05$).

[C]These chickens also had LL.

[D]Two of these chickens also had LL.

endogenous avian retroviruses (ev/J, EAV-HP and Line 0 *env*-like) (2,3,24,25). Although ALV-J have an extended host range and are able to infect domestic fowl, red jungle fowl, Sonnerat's jungle fowl, and turkeys (21), in commercial poultry ALV-J induced tumors have mostly been associated with meat-type chickens (9) and only rarely reported in egg-type chickens (31). We have recently described the isolation of an ALV from commercial egg-type chickens suffering from myeloid leukosis, and the virus was initially thought to be an ALV-J isolate base on PCR analysis with primers specific for the viral LTR. However, sequence analysis of the viral envelope indicated that the virus belongs to subgroup B (11). Sequence characterization of the SU region (gp85) of the viral glycoprotein indicates the virus belongs to subgroup B (96.8% identity), while the TM portion (gp37) of the viral envelope protein presents the highest identity (98.5%) with that of subgroup E. In addition, the 3′ UTR of the AF115-4 isolate contained the E element (also known as F2 or XSR), which has been described only in ALV-J (2) and Rous sarcoma virus (23,28). The E element is about 150 bp long and is located downstream of direct repeat 1 region (DR1) within the 3′ UTR (2). Although the function of the E element has not yet been determined, it has been shown that it has binding sites for the transcription factor c/EBP (22) and might also function as an enhancer. Most ALV-J isolated thus far posses the E element; however, there are reports of isolates where part or the entire E element is missing, indicating that it is not essential for ML induction by ALV-J (7). Interestingly, the 3′ UTR of AF115-4, starting from the stop codon of the *env* gene, is very similar to that of 5701A, an ALV-J recombinant virus that posses a subgroup A gp85 and a subgroup E gp37 gene (16). In this case the recombination took place between an ALV-J isolate and a defective endogenous recombinant virus that carries a subgroup A envelope (RAV0A1), present in the transgenic chicken line *alv6* (6). These data suggest that either the gp37 coding region of subgroup E viruses or the 3′ UTR upstream of the DR1 region are hot spots for recombination between ALV-J and other exogenous and endogenous viruses. To determine if the samples obtained from affected chickens had a mixture of subgroups B and J viruses, the host range of the AF115-4 isolate was determined using cells resistant to infection by subgroups B (C/B), E (C/E), or J (C/J) (Table 1). No virus was detected in C/B cells, suggesting that recombination took place in chickens different from those submitted for diagnosis. However, based on these experiments we cannot rule out that a wild-type ALV-B could be present in the samples examined.

Inoculation of the experimental chicken line $15I_5 \times 7_1$, with AF115-4, resulted in the induction of LL and not the expected ML (Table 3). The discrepancies observed between the kind of tumors induced by AF115-4 in commercial layer flocks and the experimental $15I_5 \times 7_1$ chicken line could be because of the high susceptibility of $15I_5 \times 7_1$ chickens to LL (1). Interestingly, similar results were obtained with seven different parental lines of commercial White Leghorn layer flocks (18). Since the oncogenic potential of retroviruses is determined by tissue-specific virus infection and replication, by tissue-specific viral promoters and/or enhancers, and by cell-specific enhancer binding proteins (4), it is possible that the commercial layer flock from where the AF115-4 virus was isolated is more susceptible to ML. However, since we cannot rule out the possibility of a wild-type ALV-B being present in the viral inoculum used for the pathogenesis studies, it is possible that the tumors observed were induced by this and not the recombinant virus. Further studies using recombinant viruses generated from infectious molecular clones will aid in clarifying this issue.

REFERENCES

1. Bacon, L. D., H. D. Hunt, and H. H. Cheng. A review of the development of chicken lines to resolve genes determining resistance to diseases. Poult. Sci. 79:1082–1093. 2000.

2. Bai, J., L. N. Payne, and M. A. Skinner. HPRS-103 (exogenous avian leukosis virus, subgroup J) has an env gene related to those of endogenous elements EAV-0 and E51 and an E element found previously only in sarcoma viruses. J. Virol. 69:779–784. 1995.

3. Benson, S. J., B. L. Ruis, A. M. Fadly, and K. F. Conklin. The unique envelope gene of the subgroup J avian leukosis virus derives from ev/J proviruses, a novel family of avian endogenous viruses. J. Virol. 72:10157–10164. 1998.

4. Brown, D. W., B. P. Blais, and H. L. Robinson. Long terminal repeat (LTR) sequences, env, and a region near the 5′ LTR influence the pathogenic potential of recombinants between Rous-associated virus types 0 and 1. J. Virol. 62:3431–3437. 1988.

5. Coffin, J. M. Structure and classification of retroviruses. In: The retroviridae, vol. 1. J. A. Levy, ed. Plenum Press, New York. pp. 19–43. 1992.

6. Crittenden, L. B., and D. W. Salter. A transgene, alv6, that expresses the envelope of subgroup a avian leukosis virus reduces the rate of congenital transmission of a field strain of avian leukosis virus. Poult. Sci. 71: 799–806. 1992.

7. Cui, Z., Y. Du, Z. Zhang, and R. F. Silva. Comparison of Chinese field strains of avian leukosis subgroup J viruses with prototype strain HPRS-103 and United States strains. Avian Dis. 47:1321–1330. 2003.

8. Elena, S. F., R. F. Miralles, J. M. Cuevas, P. E. Turner, and A. Moya. The two faces of mutation: extinction and adaptation in RNA viruses. Biochem. Mol. Biol. Int. 49:5–9. 2000.

9. Fadly, A. M., and L. N. Payne. Leukosis/sarcoma group. In: Diseases of poultry, 11th ed. Y. M. Saif, H. J. Barnes, J. R. Glisson, A. M. Fadly, L. R. McDougald, and D. E. Swayne, eds. American Association of Avian Pathologists, Kennett Square, PA. pp. 465–516. 2003.

10. Fadly, A. M., and E. J. Smith. Isolation and some characteristics of a subgroup J–like avian leukosis virus associated with myeloid leukosis in meat-type chickens in the United States. Avian Dis. 43:391–400. 1999.

11. Gingerich, E., R. E. Porter, B. Lupiani, and A. M. Fadly. Diagnosis of myeloid leukosis induced by a recombinant avian leukosis virus in commercial white leghorn egg laying flocks. Avian Dis. 46:745–748. 2002.

12. Hu, W. S., and H. M. Temin. Genetic consequences of packaging two RNA genomes in one retroviral particle: pseudodiploidy and high rate of genetic recombination. Proc. Natl. Acad. Sci. USA 87:1556–1560. 1990.

13. Hu, W. S., and H. M. Temin. Retroviral recombination and reverse transcription. Science 250:1227–1233. 1990.

14. Hunt, H. D., L. F. Lee, D. Foster, R. F. Silva, and A. M. Fadly. A genetically engineered cell line resistant to subgroup J avian leukosis virus infection (C/J). Virology 264:205–210. 1999.

15. Linial, M., and D. Blair. Genetics of retroviruses. In: RNA tumor viruses, 2nd ed. R. A. Weiss, H. Teich, H. E. Varmus, and J. M. Coffin, eds. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. pp. 649–783. 1982.

16. Lupiani, B., H. D. Hunt, R. F. Silva, and A. M. Fadly. Identification and characterization of recombinant subgroup J avian leukosis viruses (ALV) expressing subgroup A ALV envelope. Virology 276:37–43. 2000.

17. Lupiani, B., S. M. Williams, R. F. Silva, H. D. Hunt, and A. M. Fadly. Pathogenicity of two recombinant avian leukosis viruses. Avian Dis. 47:425–432. 2003.

18. Mays, J. K., A. R. Pandiri, and A. M. Fadly. Susceptibility of various parental lines of commercial white leghorn layers to infection with a naturally occurring recombinant avian leukosis virus containing subgroup B envelope and subgroup L long terminal repeat. Avian Dis. (In Press). 2006.

19. Nichol, S. RNA viruses. Life on the edge of catastrophe [news]. Nature 384:218–219. 1996.

20. Payne, L. N. Retrovirus-induced disease in poultry. Poult. Sci. 77: 1204–1212. 1998.

21. Payne, L. N., K. Howes, A. M. Gillespie, and L. M. Smith. Host range of Rous sarcoma virus pseudotype RSV(HPRS-103) in 12 avian species: support for a new avian retrovirus envelope subgroup, designated J. J. Gen. Virol. 73:2995–2997. 1992.

22. Ryden, T. A., M. de Mars, and K. Beemon. Mutation of the C/EBP binding sites in the Rous sarcoma virus long terminal repeat and gag enhancers. J. Virol. 67:2862–2870. 1993.

23. Schwartz, D. E., R. Tizard, and W. Gilbert. Nucleotide sequence of Rous sarcoma virus. Cell 32:853–869. 1983.

24. Silva, R. F., A. M. Fadly, and H. D. Hunt. Hypervariability in the envelope genes of subgroup J avian leukosis viruses obtained from different farms in the United States. Virology 272:106–111. 2000.

25. Smith, L. M., A. A. Toye, K. Howes, N. Bumstead, L. N. Payne, and K. Venugopal. Novel endogenous retroviral sequences in the chicken genome closely related to HPRS-103 (subgroup J) avian leukosis virus. J. Gen. Virol. 80:261–268. 1999.

26. Spencer, J. L. Progress towards eradication of lymphoid leukosis viruses: a review. Avian Pathol. 13:599–619. 1984.

27. Svoboda, J., M. Dvorak, R. Guntaka, and J. Geryk. Transmission of (LTR, v-src, LTR) without recombination with a helper virus. Virology 153:314–317. 1986.

28. Tsichlis, P. N., L. Donehower, G. Hager, N. Zeller, R. Malavarca, S. Astrin, and A. M. Skalka. Sequence comparison in the crossover region of an oncogenic avian retrovirus recombinant and its nononcogenic parent: genetic regions that control growth rate and oncogenic potential. Mol. Cell. Biol. 2: 1331–1338. 1982.

29. Venugopal, K. Avian leukosis virus subgroup J: a rapidly evolving group of oncogenic retroviruses. Res. Vet. Sci. 67:113–119. 1999.

30. Venugopal, K., L. M. Smith, K. Howes, and L. N. Payne. Antigenic variants of J subgroup avian leukosis virus: sequence analysis reveals multiple changes in the env gene. J. Gen. Virol. 79:757–766. 1998.

31. Xu, B., W. Dong, C. Yu, Z. He, Y. Lv, Y. Sun, X. Feng, N. Li, L. F. Lee, and M. Li. Occurrence of avian leukosis virus subgroup J in commercial layer flocks in China. Avian Pathol. 33:13–17. 2004.

ACKNOWLEDGMENTS

This work was supported in part by the National Research Initiative Competitive Grants Program, U.S. Department of Agriculture grant # 2004-35204-15033 (to B.L.). We thank Melanie Flesberg for technical assistance.